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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/978,333
Filing Date: October 15, 2001
Appellant(s): GLAZER, PETER M.

Patrea L. Pabst
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed September 21, 2005 appealing from the Office action mailed August 11, 2005.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief. Specifically, the brief (page 2) states that "the Board's attention is drawn to the appeal in U.S.S.N. 09/783,338 by the same inventor and addressing many similar issues."

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

The following grounds of rejection are not presented for review on appeal because they have been withdrawn by the examiner:

a) The rejection of claims 15-24 under 35 U.S.C. 112, second paragraph.

b) The rejection of claims 15-21, and 23-24 under 35 U.S.C. 102(b) as anticipated by Chan in view of the entry of the after final amendment filed 8/11/05.

c) The rejection of claim 22 under 35 U.S.C. 103(a) as being unpatentable over Chan et al in view of the entry of the after final amendment filed 8/11/05.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

Chan et al. Journal of Biological Chemistry. 1999. 274: 11541-11548.

Wang et al. Science. 1996. 271: 802-805.

Wang et al. Molecular and Cellular Biology. 1995. 15 : 1759-1768.

Barre et al. Proceedings of the National Academies of Sciences, USA. 2000. 97: 3084-3088.

Seidman et al. The Journal of Clinical Investigation. August 2003. 112: 487-494.

Zendegui et al. Nucleic Acids Research. 1992. 20: 307-314.

Agrawal et al. Proceedings of the National Academies of Sciences, USA. 1991. 88: 7595.

Hansson et al. Toxicology Letters. 1992. 64-65, Spec No. 141-148, abstract only.

Luo et al. Proceedings of the National Academies of Sciences, USA. 2000. 97: 9003-9008.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 7-12 and 15-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods for targeted recombination of a target nucleic acid molecule in vitro or ex vivo wherein the methods comprise contacting a target nucleic acid molecule or an isolated cell with a triple helix forming oligonucleotide (TFO) and a donor oligonucleotide that is complementary to a target nucleic acid sequence, forming a triple helix by hybridizing the TFO to the target nucleic acid, and recombining the donor oligonucleotide into the target nucleic acid to thereby accomplish targeted recombination of the target nucleic acid, wherein the TFO has a Kd of less than 2×10^{-7}, does not reasonably provide enablement for methods of in vivo targeted recombination using a donor nucleic acid and a TFO with a Kd of less than 2×10^{-7}. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The following factors have been considered in formulating this rejection (*In re Wands,* 858F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988): the breadth of the claims, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, the amount of direction or guidance presented, the presence or absence of working examples of the invention and the quantity of experimentation necessary.

The claims are drawn to methods of targeted recombination wherein the methods comprise providing a triple-helix forming oligonucleotide (TFO) to a target double-stranded molecule and a donor nucleic acid. The TFO has the property of having a binding affinity of Kd of 2×10^{-7} or less with the target nucleic acid. The donor nucleic acid may be tethered or unlinked to the TFO and recombines into the target nucleic acid sequence. The claims as written are inclusive of methods which are performed in vitro and methods which are performed in vivo (see page 3 of the specification). In particular, claim 10 allows for the use of the targeted recombination to alter the activity of a protein encoded by the target double-stranded nucleic acid. Claims 15-24 are inclusive of methods in which recombination produces a change in the genome of an intact human or animal. While the specification has enabled methods for in vitro and ex vivo targeted recombination using a donor nucleic acid tethered or unlinked to a TFO having a Kd of 2×10^{-7}, the specification has not enabled methods for in vivo targeted mutagenesis.

The specification exemplifies the in vitro treatment of cells and cell extracts with triple-helix forming oligonucleotides and teaches that oligonucleotides having a Kd of 2×10^{-8} or less are capable of inducing mutations in vitro at a rate of 0.2-0.8% (see Example 1 in the specification). The specification exemplifies a method in which a triple helix forming (TFO) oligonucleotide having a Kd of 3×10^{-5} (AG10) induced mutations at a frequency of .07 as compared to the frequency of .03 induced by a TFO oligonucleotide that did not show significant binding. The specification also teaches that

HeLa cell-free extracts treated with the AG30 TFO tethered to or unlinked to a SupFg1 donor fragment had a recombination frequency of 45 x 10^{-5} and 40 x 10^{-5} , respectively.

The specification teaches that in mice treated with AG30 (no donor fragment provided), mutagenesis was observed in liver, skin, kidney, colon, small intestine and lung cells at a frequency five fold that of background (see Example 7). The specification does not teach the treatment of mice with a TFO and donor fragment and does not teach the frequency at which recombination is achieved in intact animals or humans using TFOs and/or TFOs with donor nucleic acids.

While the specification has enabled methods of using TFOs and donor nucleic acids in vitro, the specification has not enabled methods for targeted recombination in vivo or methods in which targeted recombination produces changes in the genome of an intact animal or human. The specification does not provide any working examples in which a TFO and donor nucleic acid are used in combination to induce targeted recombination in an intact human or animal.

The activity of triple-helix forming oligonucleotides in vivo is unpredictable in the absence of in vivo data because the success of the therapy is dependent on adequate concentrations of the oligonucleotide reaching the desired site in vivo, hybridizing the TFO to a specific gene sequence, and recombining the donor fragment into the target gene sequence. Specifically, in vivo oligonucleotide therapies are unpredictable for the following reasons: (I) The triple-helix forming oligonucleotides and donor nucleic acids may be degraded in blood and tissues under physiological conditions and therefore may

not reach the target site in sufficient quantities to induce recombination; (ii) The ability of the oligonucleotide to be taken up by the cell is expected to be different under physiological conditions as versus tissue culture, i.e. the permeability of the cell would be different under physiological conditions and would be expected to vary with cell type; (iii) It is not clear what would be the optimum concentration of the oligonucleotide required for effective treatment, the mode of administration and the pharmacokinetics of therapy for the combination of TFOs and donor nucleic acids; (4) The ability of the oligonucleotide to form a triple helix under physiological conditions is unpredictable since the formation of the triple-helix varies significantly with oligonucleotide length, and chemical composition and is highly affected by the presence of secondary and tertiary nucleic acid structures; and (5) the ability of the donor oligonucleotide to bind to and recombine with the target nucleic acid varies significantly with the length and chemical composition of the donor oligonucleotide and the recombination process is also affected by secondary and tertiary nucleic acid structures. Therefore, for each therapeutic oligonucleotide, one must develop de novo the appropriate set of parameters for in vivo function. While the specification provides a general discussion regarding the administration of TFOs in combination with donor oligonucleotides, there is insufficient guidance provided in the specification as to how to use any particular oligonucleotides for therapeutic purposes in vivo and therefore one is left to develop de novo the appropriate techniques for selection, delivery and effective use of each therapeutic oligonucleotide.

In view of the unpredictability in the art of oligonucleotide therapeutics, the skilled artisan would not accept that results obtained in vitro are reasonably predictive of the results obtained in vivo for because the correlation between in vitro and in vivo results is not a general characteristic that can be applied to each and every pharmaceutical composition and the record has not established a universal correlation between the results obtained in vitro with triple-helix forming oligonucleotides/donor nucleic acids and the results obtained in vivo. Moreover, for triple-helix forming oligonucleotides which induce mutagenesis, the ability to use such oligonucleotides in vivo is further unpredictable because there is no means to predict a priori where a mutation or what particular mutation will occur.

Further, Wang (page 804; Science. 1996. 271: 802-805; cited in the IDS) teaches that the triple-helix forming oligonucleotides induce a scattered spectrum of mutations, rather than specific mutations. As discussed in the specification, targeted mutagenesis is performed to achieve the result of activating, inactivating or altering the activity and function of a gene. However, the process of targeted mutagenesis with triple-helix forming oligonucleotides does not result in a single, specific mutation. Rather, this process, as exemplified in the specification, results in the formation of an array of point mutations and deletions at or surrounding the region to which the oligonucleotide binds. While the donor oligonucleotide may be used to introduce a specific sequence into the target nucleic acid, the binding of the TFO also introduces an array of nonspecific mutations at the target site or adjacent thereto. For therapeutic purposes, the induction of random mutations would not provide an art recognized

acceptable means for the treatment of a disease because there is no predictable means for determining which mutations would have a null effect and which mutations may actually result in the inappropriate stimulation or inhibition of gene expression.

The teachings of Chan (Journal of Biological Chemistry. 1999. 274: 11541-11548; cited in the IDS) also highlight the unpredictability of using TFOs and donor nucleic acids to induce specific mutations. Chan (page 11548, col. 1) found that in cells treated in vitro with TFOs and a donor nucleic acid, at least 20% of the sequenced mutations included random deletions and other point mutations, which is consistent with the type of mutagenesis induced by TFOs alone. Chan states that "(w)e are currently exploring means of reducing nonspecific mutagenesis by changing these TFO parameters and by trying to improve the efficiency of the information transfer from the donor. " However, the present specification does not provide any specific guidance as to how to overcome the problem of inducing nonspecific mutations in vivo in intact humans or animals.

It is also highly unpredictable as to whether the TFO/donor oligonucleotide would be capable of inducing a sufficient amount of recombination and mutations in a sufficient quantity of cells to impart a therapeutic effect in view of the fact that the specification teaches that even in vitro such TFOs tethered to a donor fragment induce recombination at a frequency of 45×10^{-5} and TFOs not linked to the donor fragment induce recombination at a frequency of 40×10^{-5}. Moreover, in view of the cellular mechanisms for repairing mutations, it is highly unpredictable as to whether a sufficient

number of cells would maintain the mutation and pass the mutation on to secondary cells in order to allow for an effective therapy.

At the time the invention was made, it was well recognized in the art that significant experimentation would be required to practice the in vivo administration of triple helix forming oligonucleotides. For example, Wang (Molecular and Cellular Biology, 1995, p. 1767; cited in the IDS) states that "(c)learly, forming a triple helix in vitro under controlled buffer conditions is an easier task than doing so in vivo with exogenously added oligonucleotides, which must be taken up by the cells, enter the nuclei, and bind to the target site under suboptimal conditions". Wang (p. 1767) goes on to state that "(t)heoretically, targeted mutagenesis and inactivation of selected genes might also eventually have therapeutic applications. However, the general applicability of this approach will depend on the extension of the third-strand binding code and the development of nucleotide analogs so that triple helix formation is not limited to polypurine sequences." Yet, there is no specific guidance provided in the specification as to how to overcome the numerous known and unknown obstacles associated with in vivo targeted recombination and mutagenesis with TFOs and donor oligonucleotides. Additionally, as evidenced by Wang, excessive experimentation would be required to accomplish the successful in vivo application TFO/donor oligonucleotides that are capable of targeting specific sequences in vivo.

The teachings of Chan also emphasize the fact that additional research is required before TFOs tethered to donor oligonucleotides can be used in vivo. In particular, Chan concludes that "The TD-TFO approach as a method for DNA sequence

modification has the potential to be useful a research tool and **may eventually** provide the basis of a gene therapy strategy" (emphasis added).

Barre (PNAS (2000) 97:3084-3088; see abstract) emphasizes the requirement for mutagenesis to occur at a sufficient frequency in order for TFOs to be effective in vitro or in vivo. Barre (page 3088) states that "If this value is taken to represent the expected maximal frequency of mutations on an endogenous target, the proportion of targets reached by the TFO thus can be estimated as one in a thousand – a proportion too low to envision any therapeutic applications."

The unpredictability in the art of using TFOs in vivo is further highlighted by Seidman et al (The Journal of Clinical Investigation. August 2003, 112: 487-494). This reference (which is co-authored by the present inventor) teaches that "Biological applications of TFOs are compromised by fundamental considerations, as well as limitations imposed by physiological conditions....triplex formation involves conformational changes on the part of the third strand, and some distortion of the underlying duplex. Pyrimidine motif triplexes are unstable at physiological pH because of the requirement for cytosine protonation that occurs at relatively acidic pH (pKa = 4.5)...All of these factors impose kinetic barriers on triplex formation and reduce the stability of triplexes once formed" (pages 487-488). With respect to the importance of the stability of binding, Seidman (page 488) teaches "TFOs with equilibrium dissociation constants of approximately 10^{-9} M were active; those with Kd's of 10^{-6} M were not." The reference concludes that "It seems likely that recent advances in oligonucleotide chemistry have considerable potential for the development of TFOs with

robust gene targeting activity. This will require coordinated effort between chemists and biologists, but recent data suggests that this effort will be rewarded." Accordingly, the teachings of this reference indicate that even as of 2003, additional research is required before TFO/donor oligonucleotides can be used for in vivo applications.

In summary, there is insufficient guidance provided in the specification as to how to use the claimed TFOs/donor oligonucleotides for in vivo applications. The specification as filed provides only general guidelines for the administration of TFOs and donor oligonucleotides, leaving one to develop de novo the appropriate techniques for in vivo delivery and effective use of TFOs/donor nucleic acids for inducing targeted recombination in intact humans and animals. Accordingly, in view of the breadth of the claims, the recognized unpredictability in the art of targeted recombination and mutagenesis and in the art of the in vivo administration of TFO/donor oligonucleotides, and in view of the lack of working examples in the specification, it would require undue experimentation for one of skill in the art to practice the invention as broadly claimed.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 7-12 and 25 are rejected under 35 U.S.C. 102(b) as being anticipated by Chan et al (Journal of Biological Chemistry. 1999. 274: 11541-11548; cited in the IDS).

Chan et al (see, for example, page 11542) teach a method of targeted recombination wherein the methods comprise contacting a cell with a single stranded TFO tethered or unlinked to a donor oligonucleotide under conditions in which the TFO forms a triple helix with a target nucleic acid and the donor oligonucleotide recombines into the target nucleic acid. In particular, Chan teaches that treatment of cells in vitro with the combination of TFOs and donor oligonucleotides was able to mediate gene conversion or correction in the supF reporter gene, thereby altering the activity of the protein encoded thereby (see page 11545). In the method of Chan, the TFO (AG30) is single stranded, 30 nucleotides in length and comprises polypurine and polypyrimidine nucleotides (see page 11543 and Figure 2). AG30 has a Kd of 3×10^{-8} and the TFO tethered to the donor nucleic acid (A-AG30) has a Kd of 5×10^{-8} (see page 11543), and thereby has a Kd of less than 2×10^{-7}. With respect to claim 12, Chan teaches that the donor nucleic acid is 40 or 44 nucleotides in length (see page 11543). Accordingly, the method of Chan is considered to anticipate the claimed method of targeted recombination.

(10) Response to Arguments

Claims 7-12 and 15-25 are rejected under 35 U.S.C. 112, first paragraph

At page 9 of the brief, Appellants state that "(l)imitations directed to the *in vivo, ex vivo, and in vitro* applications of the method as defined by claims 7-12 have been continually read into the claims by the Examiner but are not in fact present in the claim language." This argument has been fully considered. However, it is noted that claims 7-

12 as broadly written encompass methods of targeted recombination of a nucleic acid molecule wherein the methods comprise the steps of providing a TFO and a donor nucleic acid. Since the claims do not define what the TFO and donor nucleic acid are provided to, the claims necessarily encompass providing the TFO and donor nucleic acid to a solution, to an isolated cell and to an intact human or animal. Thereby, it is clear that the claims encompass, in addition to in vitro and ex vivo methods, the in vivo administration of TFOs and donor nucleic acids to an intact organism.

In the brief, Appellants provide a discussion of the claimed invention, with the focus being on the teachings in the specification and prior art of the effective administration of oligonucleotides to mice. Appellants cite Zendegui as teaching that oligonucleotides can be administered by i.p. or intravenous injections and gain access to tissues (outside of the central nervous system) and to cell nuclei. Appellants conclude that chromosomal DNA throughout the somatic tissues of an animal can be targeted by nucleic acids. At page 10 of the brief, Appellants state that "Given the knowledge of one of ordinary skill in the art that for *in vivo* distribution of nucleic acids all that is required is injection of the nucleic acids, one of ordinary skill in the art would expect that a TFO injected into an animal would result in site-directed mutagenesis as predicted by the *in vitro* data." Appellants further point to Examples 6 and 7 of the specification as teaching that a TFO (alone) when administered to mice resulted in site-directed mutagenesis. Appellants assert that these in vivo results were predicted by the in vitro data. It is argued that since the specification teaches in vitro results for TFOs and donor nucleic acids, one would expect that the TFOs and donor nucleic acids would have the

"same degree of efficacy in animals as in cell systems." At page 13 of the brief, Appellants stated that "although the Examiner recognizes that delivery is an issue, Appellant has established that it is not insurmountable and provided evidence showing that it is possible to practice the claimed invention...The Patent Office is required to accept the truth of the Appellant's statements unless a reason to do otherwise can be substantiated."

Appellants arguments have been fully considered but are not persuasive for the following. In summary, the specification teaches:

a) the ***in vitro*** use of a TFO linked or unlinked to a donor nucleic acid to achieve targeted recombination of an **extrachromosomal** (i.e., plasmid) sequence in HeLa cell extracts (see example 2 and 3).

b) the *in vitro* use of TFOs alone to induce targeted mutageneis in cells and cell free extracts (see example 1).

c) the *in vivo* use of one TFO, AG30, alone to induce targeted mutagenesis in mice (see Examples 6 and 7).

Appellants have argued that because *in vitro* data was predictive of *in vivo* data for the AG30 TFO alone, then the *in vitro* data for TFOs and donor nucleic acids should be considered predictive of *in vivo* results.

This argument has been fully considered but is not persuasive because the methodology of inducing **mutagenesis using a TFO alone** is clearly distinct from the

methodology of the present invention which requires the use of a **TFO and donor nucleic acid to induce targeted recombination**. When used alone, TFOs act to induce targeted mutagenesis primarily by binding with sufficient affinity to a target sequence to provoke error-prone DNA repair. In the present invention, TFOs are used together with a donor nucleic acid. The donor nucleic acid recombines with a target nucleic acid, transferring its information into the target nucleic acid. The TFO helps position the donor nucleic acid and mediate the recombination event. Given the differences in the mechanisms of activity and effects, the result obtained with TFOs alone cannot be extrapolated to TFOs used in combination with donor nucleic acids.

Appellants state that the teachings in the specification and the teachings of Zendegui have established that oligonucleotides are taken up by cells and therefore are effective in vivo. Appellants also state that the present invention requires only providing a suitable TFO and donor nucleic acid and injecting the TFO and donor nucleic acid (pages 9-10 of the brief). However, simply providing and injecting TFOs and donor nucleic acids is not enough. The uptake of the TFOs/donor nucleic acids by cells is not the only issue that effects the efficacy and applicability of the presently claimed method. In order for the claimed method of in vivo targeted recombination to have a practical use, the donor nucleic acid must effectively recombine with the target nucleic acid to induce a sufficient number of mutations in the genome. The mutations must be specific and the mutations must not be corrected by the organism's repair system in order to allow for the mutations to be passed on to daughter cells. While no data has been provided for the in vivo use of TFOs/donor nucleic acids, the specification teaches that

even in vitro TFOs tethered to a donor fragment induce recombination only at a frequency of 45 x 10^{-5} and TFOs not linked to the donor fragment induce recombination at a frequency of 40 x 10^{-5}. Barre teaches that the efficiency of TFOs alone, even in 2000, was too low for therapeutic applications in an in vitro setting, let alone in in vivo settings, stating that "in the case of a preformed triple helix using exogenous plasmids with a high proportion of crosslinks (around 90%), the maximal frequency of mutations obtained was on the order of 10^{-3}... If this value is taken to represent the expected maximal frequency of mutations on an endogenous target, the proportion of targets reached by the TFO thus can be estimated as one in a thousand – a proportion too low to envision any therapeutic applications" (see page 3088). Also, the specification does not provide any data to establish that the mutations are maintained and passed on to secondary cells. In view of the cellular mechanisms for repairing mutations, it is highly unpredictable as to whether a sufficient number of cells would maintain the mutation and pass the mutation on to secondary cells in order to allow for an effective therapy. Further, the teachings in the art support the unpredictability of using TFOs/donor nucleic acids to induce specific mutations. In particular, Chan teaches that, with respect to TFOs/donor nucleic acids in vitro, 20% of the sequenced mutations were not specific. The number of random mutations induced by TFOs/donor nucleic acids in vivo is not presently known. It is also unknown as to what would be the outcome of inducing random mutations at a frequency of 20% and how this would effect the overall therapeutic benefit of treatment in a human or other organism. Additionally, it is noted that the data for the use of TFOs/donor nucleic acids set forth in the specification and in

the Chan reference was obtained using TFOs/donor nucleic acids which target extrachromosomal sequences. It has not been established that the results obtained regarding the frequency of inducing specific and nonspecific mutations in extrachromosomal DNA can be extrapolated to chromosomal DNA. Chan supports the unpredictability of applying TFOs/donor nucleic acids for target recombination of chromosomal DNA, stating that "the work presented here involves targeting an extrachromosomal SV40-based vector. Whereas such a vector provides a chromatinized target, it may not fully reflect a chromosomal gene...the effectiveness of TD-TFOs to mediate sequence changes at chromosomal sites remains to be determined" (see page 11548). Accordingly, Appellants have not established that the TFOs/donor nucleic acids can effectively induce a sufficient number of mutations in an intact organism, that the mutations are specific or that the mutations are passed on to daughter cells in order to provide a method of therapeutic benefit.

Appellants have cited Zendegui (1991) as teaching that oligonucleotides can be used effectively for in vivo therapies because oligonucleotides can be taken up by cells in vivo. However, Zendegui does not in fact teach that all oligonucleotides can be effectively used for in vivo therapies. Rather, Zendegui (page 312) teaches that "We have described the distribution of a 38 base oligonucleotide modified at the 3' end by addition of phopshopropyl amine. The information reported here, we believe, should be valid not only for the particular sequence shown in Figure 1, but also for other oligonucleotides of the same general size, base composition and end structure." Yet, the presently claimed TFOs and donor nucleic acids are not limited to the same size,

base composition and end structure as the oligonucleotide of Zendegui. Further, as discussed in the above rejection, the ability for cells to effectively take up TFOs in vivo remains unpredictable. For instance, Wang (1995) states that "(c)learly, forming a triple helix in vitro under controlled buffer conditions is an easier task than doing so in vivo with exogenously added oligonucleotides, which must be taken up by the cells, enter the nuclei, and bind to the target site under suboptimal conditions". Also, it is again noted that Chan (page 11548) emphasizes that the TFO technology currently has use only as a research tool, although it may eventually provide the basis of a gene therapy strategy.

For these reasons, it is maintained that the results obtained in vitro with TFOs/donor nucleic acids and the results obtained with one AG30 TFO alone in mice are not sufficient to establish the predictability of using TFOs in combination with donor nucleic acids in vivo for targeted recombination. The showing that TFOs/donor nucleic acids may be used in cell-free extracts to induce targeted recombination at a frequency of 40-45 x 10^{-5} is not sufficient to establish that TFOs/donor nucleic acids may be used in vivo to induce a sufficient number of specific mutations that are passed on to daughter cells and which thereby provide a therapeutic and useful benefit. The experimentation required to practice the claimed invention is extensive and undue give the fact that one must develop de novo the appropriate techniques for selection, delivery and effective use of each TFO/donor nucleic acid and one must overcome the hurdles of developing a therapy which allows for the introduction of a sufficient number of specific mutations into a target chromosomal DNA. In view of the unpredictability in

the art and lack of specific guidance provided in the specification, it is maintained that undue experimentation would be required to practice the claimed method of targeted recombination in vivo.

Claims 7-12 and 25 are rejected under 35 USC 102(b) as anticipated by Chan

In the brief, Appellants state that the present application is a continuation-in-part of 09/411,291 (now U.S. Patent No. 6,303,376), filed 10/4/1999, which is a divisional of U.S. Application No. 08/476,712, (now U.S. Patent No. 5,952,426), filed June 7, 1995. Appellants state that the present invention is entitled to priority to the '376 and '426 patents and thereby conclude that Chan is not available as prior art.

In the brief, Appellants point to specific teachings in the '376 and '426 patents as providing support for the limitations set forth in claims 7-12. Appellants arguments have been fully considered and are addressed below. However, it is first noted that a claim as a whole is assigned an effective filing date, rather than the subject matter within a claim being assigned individual effective filing dates. In the present situation, the '376 and '426 patents provide basis only for the general concept of administering a TFO in combination with a donor nucleic acid. The '376 and '426 patents do not provide basis for each of the limitations recited in claims 7-12. Thereby, it is maintained that claims 7-12 are entitled to the filing date of only the present application of October 15, 2001.

In particular, the claims require a TFO having a Kd of less than or equal to 2×10^{-7}. The brief points to column 5 and 9 and Table 1 of the '376 patent as providing support for this concept. However, column 5, lines 1-3 of the '376 patent teaches that

the Kd is less than or equal to approximately 10^{-7} and preferably the Kd is less than or equal to $2x10^{-8}$. Column 9 and Table 1 of the '376 patent teaches TFOs having a Kd of 3×10^{-5}, 3×10^{-7}, or 2×10^{-8}. Thereby, the priority applications provide support for TFOs having these individual Kds (i.e, a Kd of less than or equal to 10^{-7} or $2x10^{-8}$ and TFOs having a Kd of 3×10^{-5}, 3×10^{-7}, or 2×10^{-8}). However, the teachings of these particular Kds and the teachings of a Kd of less than or equal to 10^{-7} is not sufficient to provide support for the specific embodiment required by claims 7-12 of a TFO having a Kd of less than or equal to **2×10^{-7}.**

Additionally, the '426 and '376 patents do not provide basis for the concept of TFOs having a length that is between 10 and 60 nucleotides. In the brief, Appellants state that support for this limitation may be found in the '426 and '376 patents which disclose TFOs of 7 to 40 nucleotides and specifically exemplify TFOs of 10, 20, 30 or 57 nucleotides (see column 3, lines 66-67 and column 4, lines 1-3, and columns 7-8). This argument has been fully considered but is not persuasive. An example of a TFO of 57 nucleotides, together with a teaching that the TFO may be 7 to 40 nucleotides does not provide basis for the concept encompassed by claim 8 of TFOs of 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 58, 59 or 60 nucleotides.

Claims 7-12 further require the use of a TFO that is tethered or untethered to a donor nucleic acid. However, the '376 and '426 patents do not provide support for the concept of a TFO tethered to a donor nucleic acid. The brief addresses the limitation of a donor nucleic acid tethered to a TFO only to the extent that this limitation is recited in claim 9. However, it is noted that each of the claims encompass methods in which the

donor nucleic acid is tethered to the TFO. In the brief, Appellants point to column 1 to 2 of the '376 patent as teaching that TFOs are useful alone or linked to reactive moieties. Appellants conclude that it would thereby be clear that the DNA fragment could also be linked to the TFO. This argument is not persuasive because the '376 patent does not suggest linkage of the TFO to any reactive moiety and does not specifically teach linkage of the TFO to the donor nucleic acid. Rather, col. 1-2 of the '376 patent states that "Site specific cleavage of DNA has been achieved by using triplex forming oligonucleotides linked to reactive moieties such as EDTA-Fe(II) or by using triplex forming oligonucleotides in conjunction with DNA modifying enzymes....Triplex forming oligonucleotides linked to intercalating agents such as acridine, or to cross-linking agents, such as p-azidophenacyl and psoralen, have been utilized, but only to enhance the stability of the triplex binding." A reading of these passages would not lead one to the conclusion that the '376 patent intended to disclose TFOs linked to a donor nucleic acid. The "reactive moieties" of EDTA-Fe(II) and modifying enzymes were attached to TFOs for the purpose of site specific cleavage of the DNA, and the intercalating agents were attached to the TFOs in order to enhance the triplex binding. On the other hand, the donor nucleic acids are used in conjunction with TFOs for the purposes of stimulating homologous recombination of the donor nucleic acid into the target region. Further, the statements in the '376 patent regarding a review of methods which were used in the prior art do not provide support for the concept of applying this methodology to the TFO method disclosed therein in which the TFO is used in conjunction with a donor nucleic acid. In summary, the cited passages in the '376 patent of prior art

methods of linking TFOs to cleavage agents and intercalating agents do not provide basis for the concept of linking TFOs to donor nucleic acids.

With respect to claims 12 and 25, the '426 and '376 patents also do not provide basis for the concept that the donor nucleic acid is at least 30 nucleotides in length or is between 10 to 40 nucleotides. There are no teachings in the '426 or '376 patents regarding the length of the donor nucleic acid. Further, the teachings in the '426 and '376 patents regarding the lengths of TFOs do not apply to the donor nucleic acid since the TFOs are distinct from the donor nucleic acids with respect to their functional activities. It is noted that the brief does not specifically address this limitation.

Accordingly, it is maintained that the present invention is entitled to the filing date of October 15, 2001 and thereby Chan is in fact prior art to the claimed invention.

(11) Related Proceeding(s) Appendix

Copies of the court or Board decision(s) identified in the Related Appeals and Interferences section of this examiner's answer are provided herein.

Appellants have stated that the appeal in U.S.S.N. 09/783,338 addresses "similar issues" as those set forth in the present application. However, it is noted that the claimed method of the '338 application is clearly distinct from the presently claimed method and thereby the issues for each application are also clearly distinct from one another. The claims in the '338 application are limited to methods of site directed mutagenesis using a TFO having a mutagen incorporated therein. On the other hand, the presently claimed invention is directed to a method of targeted recombination using a TFO and a tethered or untethered donor nucleic acid. In the '338 application, the TFO

acts to deliver a mutagen, such as psoralen, to the site at which a mutation is to be induced by the mutagen. In contrast, in the presently claimed method, the TFO helps to position the donor nucleic acid, allowing the donor nucleic acid to recombine with a target nucleic acid and transfer its information into the target nucleic acid. Thereby, the methodologies differ with respect to their reagents (i.e., a TFO having a mutagen incorporated therein in '338 vs a TFO tethered or untethered to a donor nucleic acid in the present invention) and with respect to their mechanisms of activity and effect.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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